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Effects of stress activated protein kinases on the expression of EST3 gene that encodes telomerase subunit in *Saccharomyces cerevisiae*

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Abstract: The EST3 gene of *Saccharomyces cerevisiae* encodes one of the essential subunits of telomerase enzyme. Expression of the EST3 gene is regulated at the translation level by +1 programmed ribosomal frameshift (PRF). It is known that physiological stress affects telomere length. In this study, we have investigated the effects of different types of stresses and stress activated protein kinases on the frameshift rate in EST3 gene. PRF rate of EST3 gene was measured as 13% in the normal growth conditions in the wild type cells. But, the PRF rate of EST3 in the wild type strain grown in glucose limited conditions decreased more than 6-fold. Contrary to glucose limitation, osmotic stress increased frameshift rate from 13% to 25%. Amino acid starvation and boron stress also activate PRF rate by 2-fold in EST3 in a Gcn2 dependent manner. When the PRF rate was analysed in *gcn2* and *snf1* mutants, frame shift rate of EST3 was approximately 6% in normal growth conditions. It seems that the basal level expression of EST3 is highly dependent on the Gcn2 kinase complex, indicating that Gcn2 might have a significant function in connecting the stress signals to biosynthesis of the full-length Est3 peptide. This regulation might connect the biosynthesis of functional telomerase and telomere replications to cell physiology through stress activated protein kinases.

Key words: frameshift; amino acid starvation; stress activated protein kinases; telomerase.

Abbreviations: 3-AT, 3-amino 1,2,4 triazole; ASC1, absence of growth suppressor of cyp1; EST, ever shorter telomer; eEF, elongation factor; FF, frame fusion; FS, frameshift; GCN, general control non-derepressed; HOG, high osmolarity glycerol; MAPK, mitogen activated kinase; ONPG, 2-nitrophenyl β-D-galactopyranoside; PRF, programmed ribosomal frameshift; RCK, radiation sensitivity complementing kinase; SAPK, stress activated protein kinase; SDS, sodium dodecyl sulphate; SNF, sucrose non-fermenting; STM1, suppressor of ToM1; TLC1, telomerase component 1; Ty3, transposon yeast-3.

Introduction

The protection of telomere lengths and telomere replication is the crucial part of the chromosome integrity and cellular functions (Wellinger & Zakian 2012). It has been shown that the telomere length and the telomerase activity are regulated by large set of genes depending on the growth conditions in *Saccharomyces cerevisiae* (Harari & Kupiec 2014). Romano et al. (2013) investigated the effects of dozens of stress inducing agents on telomere length. They have shown that certain stress agents (such as acetic acid and ethanol) significantly increase the telomere length, while other stresses (caffeine, high temperature, hydroxyurea) lead to telomere shortening (Romano et al. 2013). Their results indicated that different types of environmental stresses affect telomere length through different genes (Romano et al. 2013). In accord with telomere length, some stress agents also increase or decrease telomerase activity (Kupiec & Weisman 2012; Kepinska et al. 2015).

Telomerase enzyme complex is composed of 3 different peptide and telomerase RNA in *S. cerevisiae*. Est1 (ever shorter telomere) and Est3 function as regulatory subunits of telomerase complex. Est2 is the catalytic subunit with reverse transcriptase like activity. TLC1 RNA functions as RNA template in telomere replication (Taggart & Zakian 2003; Wellinger & Zakian 2012). Mutations in any one of the EST genes results in progressive loss of telomeric DNA (Lundblad & Szostak 1989; Wellinger & Zakian 2012).

Expression of EST3 is regulated by +1 programmed ribosomal frameshift (PRF) (Morris & Lundblad 1997). It has been shown that EST3 mRNA encodes 2 peptides, one of them being 93 amino acid long truncated peptide with no known function. Translation of the full-length, 181 amino acid long functional Est3 peptide, requires +1 PRF (Morris & Lundblad 1997). Molecular mechanisms of PRF in EST3 are well documented (Taliaferro & Farabaugh 2007). It has been shown that the CUU AGU U sequence on EST3 mRNA (frameshift site) and the 27 nucleotide long stimulatory mRNA sequence (3' to frameshift site) are sufficient for

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the PRF in EST3 (Taliaferro & Farabaugh 2007). It has also been shown that CUU decoding P-site tRNA (tRNA-Leu-UAG) slips one nucleotide upon pause resulting from slow decoding A-site tRNA (tRNA-Ser-GCU). Once tRNA slippage occurs, A-site is then decoded by GUU decoding tRNA (tRNA-Val-IAC), and translation resumes in +1 frame, giving full length and functional Est3 peptides (Taliaferro & Farabaugh 2007). A stimulatory sequence, downstream region 3' to frameshift site, affects PRF rate though its exact role in the PRF is not known currently (Taliaferro & Farabaugh 2007). Advani et al. (2013) provided evidence indicating that expression of EST1 and EST2 is also regulated by −1 ribosomal frameshift. They have identified several slippery sequences on the EST1 and EST2 mRNAs, which are a potential −1 ribosomal frameshift sites for these mRNAs. Hence, they have suggested that telomeres in yeast are globally controlled by programmed ribosomal frameshift and nonsense-mediated mRNA decay pathway.

Stress activated protein kinases (SAPKs) regulate various metabolic pathways to coup with environmental stimuli (Engelberg et al. 2014). Snf1, Hog1 and Gcn2 are the major stress activated protein kinases in *S. cerevisiae*. Hog1 is activated mainly by osmotic stress, and activation of Hog1 results in increased glycerol biosynthesis (De Nadal & Posas 2010; Duch et al. 2012). Osmotic stress also leads to translational down regulation in yeast proteome (Teige et al. 2001; Warringer et al. 2010). Snf1 is a multi-functional protein kinase in yeasts and upon activation, it regulates diverse metabolic pathways in response to glucose starvation, autophagy, heat shock, oxidative and osmotic stress (Hedbacker & Carlson 2008). Snf1 is also activated by physiological stresses by its upstream kinases Sak1, Elm1 and Tos3 (Hong & Carlson 2007). Gcn2 is another protein kinase that affects a wide range of metabolic events in yeasts. Initially, it has been reported that Gcn2 is activated by amino acid starvation and regulates translation initiation through eukaryotic initiation factor-2 phosphorylation in *S. cerevisiae* (Wek et al. 1994). However, later, it was shown that Gcn2 also regulates many metabolic pathways, such as oxidative stress, and nutrient limitations. It is also being activated by other stresses, such as boron stress (Uluisik et al. 2011; Castilho et al. 2014). It has been documented that, in addition to cytoplasm, Gcn2 is present in translation elongation complex and associates with large subunit of ribosomes (Visweswaraiah et al. 2011). Gcn2 form complexes with Gcn1 and Gcn20 (Martonet al. 1997). Gcn1 and Gcn20 function as effector molecules for Gcn2 phosphorylation (Garcia-Barrio et al. 2000). Gcn1 mediates binding of Gcn2 to ribosomes (Sattlegger & Hinnebusch 2005). The cross-regulatory interactions among these SAPKs have been reported. Hence these SAPKs also function in an interdependent manner (Cherkasova et al. 2010; Shashkova et al. 2015).

The purpose of this research was to investigate if the various stress conditions have any effect on the PRF rate in EST3. Hence, PRF rate of EST3 was investigated in yeast cells that were exposed to stress inducing growth conditions. The functions of SAPKs Gcn2, Snf1 and Hog1 in the PRF efficiency of EST3 were analysed in the normally grown and stress-inducing growth conditions. Our results indicated that PRF rate of EST3 can be activated or repressed several folds by various stress conditions, and Gcn2, Snf1, and Hog1 are involved in the stress dependent control of PRF efficiency in EST3.

Material and methods

Yeast strains and plasmids

BY4741 based wild-type (MATa, *his3Δ1; leu2Δ0; met15Δ0; ura3Δ0*) and mutant strains of *S. cerevisiae* used in this research were purchased from EUROSCARF yeast collection. Their accession numbers and relevant genotypes are as follows: Y00000 (wild-type), Y02724 (Δ*hog1*), Y05157 (Δ*rck2*) Y04546 (Δ*gcn1*), Y05688 (Δ*gcn20*), Y03642 (Δ*gcn2*), Y14311 (Δ*snf1*). These yeast strains are isogenic other than indicated pathway's mutations. *S. cerevisiae* strain Σ1278b (MATa, *ura3-52*) was used as the wild-type strain in amino acid starvation experiments (Zurita-Martinez & Cardenas 2005).

PRF efficiency of EST3 was measured using a reporter plasmid that contains EST3-frameshift (EST3-FS) or EST3-frame fusion (EST3-FF) sites fused to *E. coli* lacZ genes. Structural features of EST3-FS (pDT265) and EST3-FF (pDT261) reporter plasmids were given in details previously (Taliaferro & Farabaugh 2007). These plasmids are 2-μm-*URA3*-based shuttle vectors and they are based on the previously described expression vector pANU7 (Sundararajan et al. 1999). In pDT265, EST3 gene frameshift site (CTT-AGT-T) is fused to the *E. coli* lacZ gene in the +1 reading frame. This creates the frameshift reporter plasmid. In this vector, translation of the lacZ fusion protein depends on the occurrence of frameshift event in the +1 direction at the 5'-CUU-AGU-U-3' sequence of mRNA. In EST3-FF plasmid "C" nucleotide in CTT-AGT-T is deleted to create in-frame fusion with downstream LacZ sequence; hence translation of the lacZ fusion protein does not require a frameshift event (Taliaferro & Farabaugh 2007).

Yeast cells were cultured in yeast extract peptone dextrose medium for transformation. pDT265 and pDT261 plasmids were transformed into the *S. cerevisiae* cells using the lithium acetate-polyethylene glycol method (Rose et al. 1990). It is known that 2-μm-*URA3*-based plasmids can be stably maintained in different yeast transformants when grown in selective growth conditions (Liao et al. 1987).

Growth conditions

Yeast transformants were grown in liquid synthetic complete medium without uracil (Sc-Ura) supplemented with 2% glucose as described previously (Rose et al. 1990). All experiments were done in triplicates and assays were repeated at least once. For activation of high osmolarity-glycerol (HOG) pathway and Hog1 kinase, yeast transformants were first grown to saturation over-night in 5 mL of liquid Sc-Ura media supplemented with 2% glucose, and then diluted to OD_{600}: 0.1–0.15 in 10 mL of fresh Sc-Ura+2% glucose media. Yeast transformants were grown to early logarithmic stage (OD_{600}: 0.7) and then were divided into two aliquots (5 mL each). Sterile NaCl was added to 0.8 M final concentration to the one set of yeast cultures and further incubated for 5 hours at 30 °C, in incubator shaker (140 rpm). At the

ends of the incubation period, yeast cultures were harvested for β-galactosidase assays.

For activation of Gcn2 kinase with boric acid treatment, yeast transformants were grown to early logarithmic stage as explained above. Sterile boric acid solution was added to yeast cultures to 50 mM final concentration, and then yeast cultures were further incubated for 5 hours as above described manner (Uluisik et al. 2011).

Gcn2 is also activated by 3-amino 1,2,4 triazole (3-AT) treatment of yeast cells. 3-AT is an inhibitor of HIS3 gene product and activates Gcn2 kinase by increasing uncharged tRNA levels in the yeast cells (Marton et al. 1997). In order to activate Gcn2 by 3-AT treatment, *S. cerevisiae* Σ1278 transformants were grown in YNB (6.7 g yeast nitrogen base without amino acids) + 2% glucose medium to logarithmic stage first. Then, filter sterilized 3-AT was added to 10 mM final concentration to *S. cerevisiae* Σ1278b strains. Yeast transformants were further incubated for additional 5 hours and then harvested for β-galactosidase assays.

For glucose starvation and activation of Snf1 kinase, yeast transformants (except Δ*snf1* mutant) were cultivated in Sc-Ura medium supplemented with 2% glycerol and 2% lactate as carbon sources. Since Δ*snf1* mutant *S. cerevisiae* strains cannot grow in glycerol lactate, first, transformants of Δ*snf1* yeast strain were grown in Sc-Ura+2% glucose medium to log stage. Then yeast transformants were washed with 10 mL of sterile distilled water twice, and shifted to 5 mL Sc-Ura medium supplemented with 2% glycerol and lactate. Yeast cultures were further incubated for at least 5 hours in Sc-ura+2% glycerol lactate medium at 30°C incubator shaker, and then harvested for β-galactosidase assays.

Enzyme assays

Expression levels of EST3-lacZ gene fusions in pDT265 and pDT261 vectors were quantitatively measured by β-galactosidase assays in triplicates (Guarente 1983). After the growth periods, yeast transformants were harvested and re-suspended in 200 μL of break buffer. To obtain permeabilized yeast cell lysates, 20 μL of 0.1% SDS and 20 μL chloroform were added to yeast suspension and vigorously vortexed for 1 min. These yeast cell lysates were used to determine β-galactosidase activities expressed from EST3 gene fusions in yeast strains. β-Galactosidase units are given in nmol of ONPG (2-nitrophenyl β-D-galactopyranoside) cleaved per minute per mg of protein in permeabilized yeast cells. Protein concentrations in the permeabilized yeast lysates were determined by the Lowry assay (Lowry et al. 1951). Frameshift rates of EST3 in yeast strains were calculated by dividing the β-galactosidase activities expressed from EST3-FS vector to β-galactosidase activities expressed from EST3-FF vector in the yeast strains that are grown in normal or stress inducing growth conditions. The results were multiplied by 100 to obtain percentage FS ratio.

Results

Stress conditions affect the frameshift rate in EST3

To investigate if the PRF rate in *EST3* is be affected by different stresses, such as glucose limitation, amino acid starvation or osmotic stress, PRF rate of EST3 was analysed in the wild-type *S. cerevisiae* strain. The PRF rate for EST3 in the wild-type yeast cells that grown in normal growth conditions were measured as 13% (Table 1). However, when 0.8 M NaCl was applied to growth medium to induce osmotic stress and activate Hog1, the PRF rate of EST3 increased to 23%. It is known that boron stress activates Gcn2 kinase in yeast (Uluisik et al. 2011). Adding boric acid to 50 mM also activates PRF rate up to 25% in EST3 (Table 1). In addition to these two stress conditions, yeast transformants were also subjected to glucose limitations to activate Snf1 kinase. Hence, glycerol and lactate (2% each) were included in the growth medium as the only carbon sources. Growth of yeast transformants in glycerol/lactate medium resulted in a 6-fold decrease (from 13% to 2%) in the PRF rate of EST3 (Table 1).

Table 1. Stress conditions affects PRF efficiency in EST in wild type *S. cerevisiae* cells.

Growth conditions	Frameshift rates ± SD (%)
Normal growth	13 ± 1
+0.8 M NaCl	23 ± 1
+50 mM Boric acid	25 ± 0.3
2% Glycerol/lactate	2 ± 0.3

Table 2. Gcn complex controls PRF efficiency in EST translation.

Growth conditions	Frameshift rates ± SD (%)		
	Δ*gcn1*	Δ*gcn20*	Δ*gcn2*
Normal growth	8 ± 0.2	7 ± 0.3	5 ± 1
+0.8 M NaCl	10 ± 0.3	11 ± 1	7 ± 1
+50 mM Boric acid	10 ± 0.5	12 ± 0.3	11 ± 0.3

Gcn2 is essential for the regulation of EST3-FS rate

Gcn2 is a multi-functional protein kinase (Castilho et al. 2014). It is activated mainly by uncharged tRNA binding, resulting from amino acid starvation. However, it has recently been shown that it is activated by osmotic stress and also by boron stress (Uluisik et al. 2011). Moreover, Gcn2 is also present in translation elongation complex and is associated with large subunit of ribosomes (Visweswaraiah et al. 2011). To see if the Gcn2 is involved in the activation of PRF rate, EST3 PRF rate was analysed in the Δ*gcn2* mutant yeast strain grown in normal and in stress inducing growth conditions. PRF rate of EST3 was measured as 5% in normal growth conditions in the Δ*gcn2* mutant strain (Table 2). This PRF rate of EST3 was nearly 2-fold lower than the wild-type yeast strain, which indicates that Gcn2p is involved in the normal level expression of EST3. When Δ*gcn2* mutant strain was subjected to osmotic or boron stress, PRF rate of EST3 was determined as 7% and 11%, respectively. It appears that there is an increase in the PRF rate in Δ*gcn2* mutant strain, when it is subjected to stress conditions. Nonetheless, PRF rates of EST3 in stress conditions are still 2 to 3-fold lower than the PRF rates of the wild-type yeast strains (Tables 1 and 2).

Gcn2 can interact with its effector proteins Gcn1 and Gcn20. To show if Gcn1 and Gcn20 are involved

Table 3. Amino acid starvation activates PRF efficiency in EST3 in *S. cerevisiae* Σ1278b strain.

Growth conditions	Frameshift rates ± SD (%)
Normal growth	14 ± 1
+10 mM 3-AT	21 ± 1
+50 mM Boric acid	22 ± 1

Table 4. Hog1 involved in the activation of PRF efficiency in EST in response to high osmolarity.

Growth conditions	Frameshift rates ± SD (%)	
	Δ*hog1*	Δ*rck2*
Normal growth	11 ± 0.5	11 ± 0.3
+0.8 M NaCl	14 ± 2	22 ± 1
+50 mM Boric acid	21 ± 1	29 ± 1

in the activation of PRF rate, we also measured EST3 PRF rate in the Δ*gcn1* and Δ*gcn20* mutant *S. cerevisiae* strains. The basal level of EST3 PRF rates in these mutants under normal growth conditions were 7–8%, which is slightly higher than the PRF rate in Δ*gcn2* mutant but lower than the wild-type (13%) level PRF rate (Table 2). Treatment of Δ*gcn1* mutant with 0.8 M NaCl or 50 mM boric acid did not activate EST3 PRF at significant levels. However, in Δ*gcn20* mutant, EST3 PRF rate increased from 7% to 11–12% when osmotic or boron stress was applied. These results indicated that Gcn2, which is associated with Gcn1 and Gcn20, is essential for the optimal level frameshift in EST3 in normal growth conditions and also for stress dependent activation of PRF in *S. cerevisiae*.

It is well known that the uncharged tRNA, resulting from the amino acid starvation, activates Gcn2. Treatment of wild-type yeast cultures with 3-AT, competitive inhibitor of *HIS3* gene product, leads to amino acid starvation, and increases uncharged tRNA level in yeast. Since the *S. cerevisiae* BY4741 strain is histidine auxotroph, 3-AT cannot be applied to these yeasts. In order to show that activation of Gcn2 with 3-AT treatment also affects EST3 PRF rate, we have used *S. cerevisiae* Σ1278b yeast strain, which is prototroph for histidine biosynthesis. EST3-FS and ES-FF expression vectors transformed to *S. cerevisiae* Σ1278b. Yeast transformants were grown to logarithmic stage and then treated with 10 mM 3-AT for 4 hours. EST3 PRF rate was measured as 21% in amino acid starved yeast transformants as opposed to 14% in normally grown ones (Table 3). Boron treatment that activates Gcn2, also increases EST3 PRF rate from 14% to 22% in this yeast strain (Table 3).

Hog1 is involved in the regulation of EST3 frameshift frequency

We have shown that the osmotic stress activates PRF rate in EST3 in the wild-type yeast cells. Hog1 is activated in response to high osmolarity in *S. cerevisiae* (Saito & Posas 2012). To show if the protein kinase Hog1 is involved in the activation of EST3 PRF rate in response to osmotic stress, PRF rate was analysed in the Δ*hog1* mutant yeast strains. When grown in normal growth medium, EST3 PRF rate was measured as 11% in the Δ*hog1* mutant yeast. When osmotic stress was applied to Δ*hog1* mutant strain, PRF rate in EST3 was not activated and remained essentially at the basal level (Table 4). When it is activated by osmotic stress, Hog1 also activates Rck2. Activated Rck2 is involved in the translational regulation in response to osmotic stress (Tiege et al. 2001). Hence, the effect of Rck2 on the PRF rate in EST3 was also analysed. The PRF rate of EST3 in Δ*rck2* mutant yeast was determined as 11% in normal growth conditions, which is not much different than the wild-type yeast strain. Growth of Δ*rck2* mutant yeast in osmotic stress inducing conditions, the PRF rate of EST3 increased to 22%, indicating that Rck2 is not essential for the regulation PRF efficiency in EST3 (Table 4). It is known that Hog1 is also activated by multiple stress conditions, and there is a cross-talk between Hog1 and Gcn2 (Rodriguez-Hernandez et al. 2003; Lawrence et al. 2004). Hence, we have also tested if boric acid application (boron stress) operates through the Hog1 and Rck2 kinases in the regulation of PRF rate. Growth of Δ*hog1* and Δ*rck2* mutants in boric acid applied medium resulted in PRF rates of EST3 21% and 29% in these mutants, respectively (Table 4). All together, these results suggest that the Hog1 kinase is involved in the activation of PRF rate in EST3 in response to osmotic stress, but Rck2 is not.

Snf1 is involved in the regulation of EST3 frameshift rate in response to various stresses

We have shown that the growth of the wild-type yeast cells in glucose limitation resulted in a 6-fold decrease in the PRF rate of EST3 (Table 1). It is known that the protein kinase Snf1 is activated when the yeast cells are grown in glucose limited medium. Snf1 is also activated by other stress conditions through its upstream kinases (Hong & Carlson 2007). Therefore, the PRF rate of EST3 was analysed in the Δ*snf1* mutant yeast grown in normal and stress induced growth conditions. Basal level PRF rate in EST3 was 13% in the wild-type yeast, but it decreased to 7% in Δ*snf1* mutant yeast grown in normal growth conditions. PRF rate in EST3 remains nearly at the basal level (5%) when the Δ*snf1* mutant strain cultivated in the growth medium containing glycerol/lactate as sole carbon sources (Table 5). Next, we have analysed the effects of osmotic stress and boron stress on the PRF rate in Δ*snf1* mutant. Frameshift rate increased to 16% in osmotic stress applied Δ*snf1* mutant (Table 5). This frameshift rate is approximately 2-fold higher than the basal level in Δ*snf1* mutant, but it is noticeably lower than the frameshift rate (23%) in wild-type yeast strain that was grown in osmotic stress induced conditions. Boron stress increased frameshift rate of EST3 from 7% to 19% in Δ*snf1* mutant (Ta-

Table 5. Snf1 involved in the activation of PRF efficiency in EST in response to various stresses.

Growth conditions	Frameshift rates ± SD (%)
Normal growth	7 ± 0.5
2% Glycerol/lactate	5 ± 1
+0.8 M NaCl	16 ± 1
+50 mM Boric acid	19 ± 1

ble 5). These results suggested that Snf1 is essential for the basal level frameshift rate and also is involved in the down-regulation of EST3 frameshift rate in glucose limited growth conditions. It seems that the osmotic stress and boron stress activated frameshift rate in EST3 are mostly independent from Snf1 kinase.

Discussion

Telomere length homeostasis is the key factor for the stability of eukaryotic genomes (Wellinger & Zakian 2012). Genome-wide studies on the telomere biology indicate that nearly 400 genes, also known as telomere maintenance genes, affect telomere length in *S. cerevisiae* (Ungar et al. 2009; Harari & Kupiec 2014). It has been also shown that various stress factors increase or decrease telomere length in *S. cerevisiae* (Romano et al. 2013). EST3 is the critical regulatory subunit of the yeast telomerase complex (Lundblad & Szostak 1989; Tuzonet al. 2011). Biosynthesis of the functional Est3 protein requires +1 ribosomal frameshift. Molecular mechanism of PRF in EST3 was explained previously (Morris & Lundblad 1997; Taliaferro & Farabaugh 2007).

It is known that various environmental stresses affect telomere length in *S. cerevisiae* (Romano et al, 2013). Environmental stresses also activate different protein kinases, commonly known as SAPKs (Engelberg et al. 2014). In *S. cerevisiae*, Hog1, Snf1, and Gcn2 are the well-known examples of SAPKs. In this study, we have shown that environmental stresses also affect PRF rate in EST3, and SAPKs Hog1, Snf1 and Gcn2 are involved in the stress-dependent regulation of PRF rate in EST3.

Glucose limitation decreased PRF rate by 5-fold in EST3 in the wild-type yeast cells, but not in the Δ*snf1* mutant, indicating that functional Snf1 is involved in the regulation of PRF rate in response to glucose limitations. We think that these SAPKs have multiple targets on the translation elongation complex that ribosomal frameshift takes place. One of the potential targets of SAPKs could be elongation factor-1 (eEF1) complex, which delivers charged tRNAs to ribosomal A-site. eEF1 complex has β-subunit (EFB1, EF1beta) that is required for nucleotide exchange to regenerate eEF1alpha. This subunit is also involved in and facilitates binding aminoacyl-tRNA to the ribosomal A-site. EF1beta is a phosphoprotein and has multiple phosphorylation sites (Swaney et al. 2013). eEF1alpha also has potential phosphorylation sites that can be targeted by one of the SAPKs. Hence, post translational modification of one of subunits of the eEF1 complex could easily change its structure, which might lead to significant changes in translational fidelity. Previously, it has been shown that mutations in eEF1alpha changes the frameshift rate in transposon yeast-3 (Ty3), which also takes place as +1 direction (Farabaugh & Vimaladithan 1998). It has been proven that Gcn2p specifically interacts with eEF1alpha in yeast (Visweswaraiah et al. 2011). Interactions of Gcn2p with eEF1alpha, diminish the kinase activity of Gcn2. However, whether Gcn2-eEF1alpha interaction affects the function of eEF1alpha has not been elucidated yet. Gcn2, together with its effector counterparts Gcn1 and Gcn20, is associated with elongating ribosomes. It has been suggested that Gcn2p complex monitors uncharged tRNA levels that interacts with ribosomal A-site, and then triggers global response to amino acid starvation (Visweswaraiah et al. 2011). Our results suggest that lack of activation in PRF rate in Δ*gcn* mutants in response to amino acid starvation and boron stress indicates that Gcn2 is essential both for the optimal level ribosomal frameshift under normal growth conditions and also for the stress activated induction of PRF rate in EST3. Moreover, it seems that both Gcn1 and Gcn2 are essential for the Gcn2 function in the regulation of PRF rate in EST3, since PRF rate is not activated by stress conditions in the Δ*gcn1* and Δ*gcn20* mutants.

Hog1 is a MAPK that is activated by osmotic or acid stress. Hog1 has a pivotal role in the transcriptional regulation of stress activated genes in response to high salt (such as 0.8 M NaCl) or low pH (300 mM citric acid) (Duch et al. 2012; Saito et al. 2012). It has recently been shown that Hog1 also regulates translation in yeast through its downstream target Rck2 (Teige et al. 2001; Warringer et al. 2010). Our results indicate that osmotic stress also activates PRF rate in EST3, and Hog1, but not Rck2, is involved in the activation of PRF rate in EST3. While osmotic stress activates PRF in EST3 by at least 2-fold in the wild-type yeast, it remains at same, uninduced level in Δ*hog1* and Δ*gcn2* mutant yeast. Nonetheless, boron treatment of Δ*hog1* mutant leads to 2-fold activation in the PRF rate, indicating that Gcn2 can activates PRF rate independent of Hog1 and Snf1. From this result, it is also clear that osmotic stress dependent activation of PRF rate in EST3 is dependent on Hog1 and Gcn2 complex.

Ribosome-associated proteins Asc1 and Stm1 have a significant function in the overall organization of translation complex. Both proteins are located on the mRNA binding tunnel of eukaryotic ribosomes (Nilsson et al. 2004; Ben-Shem et al. 2011). Stm1 is a multifunctional protein that is present in ribosomes and also in telomere cap complex (van Dyke et al. 2004, 2006). It has been found that the Stm1 facilitates the translation process under stress inducing conditions (van Dyke et al. 2006). Previously, we have shown that Stm1 and Asc1 (homolog of human Rack1) are required for the regulation of ribosomal frameshift in Ty3, which also

takes place as +1 frameshift (Türkel et al. 2011). It was suggested that Asc1 is the ribosome-associated protein that links various signal transduction patways directly to translation elongation complex (Nilsson et al. 2004). Later, it was also shown that Asc1 is also essential for the translational fidelity and reading frame maintenance during elongation stage of translation (Wolf & Grayhack 2015). A transient interaction between Asc1 and Gcn2 has been reported (Valerius et al. 2007). Stm1 is a phosphoprotein and targeted by Snf1 and Gcn2 (Ptacek et al. 2005; Krogan et al. 2006). Phosphorylation of Stm1 by Gcn2 and/or Snf1p might affect the EST3 mRNA topology on the A-site, which might change the specificity and also the binding efficiency of A-site tRNA. It has been also shown that the EST1 and EST2 genes that encode other essential subunits of telomerase complex, are also regulated by ribosomal frameshift (Advani et al. 2013). It is not known if the PRF rate in EST1 and EST2 will be also affected by stress-activated conditions. However, one can expect that the SAPKs might also affect the PRF rate in EST1 and EST2 even though PRF event takes place at −1 direction in these two mRNAs.

In conclusion, it is likely that Snf1, Gcn2 and Hog1 might have multiple targets on elongating ribosomes, such as EF1 complex, Stm1 and Asc1. SAPKs might act on the telomere length in an inter-dependent manner by regulating the PRF rate in EST3 *S. cerevisiae*.

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